MACROPHAGE MIGRATION INHIBITORY FACTOR AS DIAGNOSTIC AND PROGNOSTIC MARKER FOR METASTATIC ADENOCARCINOMA

FIELD OF THE INVENTION

The described invention relates to the diagnosis and prognosis of human adenocarcinoma biopsies. More specifically, this invention uses levels of macrophage migration inhibitory factor within the tumor cells as a measure of metastatic potential and kits for performing the diagnosis.

BACKGROUND OF THE INVENTION

Metastasis, the spread of cancer from a primary site to distant organs, still remains the main cause of death for most cancer patients. Despite years of research, the genetic mechanisms involved in the process are ill defined. Such information is of special importance in cancer prognosis given the uncertain course of the disease. The greatest obstacle to the successful treatment of the cancer patient continues to be the lack of sound prognostic markers, indeed cancer prognosis can not always be accurately assessed using current tumor grading techniques.

The mechanisms which regulate the growth of the cancer cell are of particular relevance to the development of strategies for the treatment of metastatic cancer. Individual patients exhibit extreme variation in cancer progression. In some patients the cancer remains localized, whereas in other the cancer metastasizes quickly. Stromal-epithelial interactions (mediated through cytokine and other growth factors) with the extracellular matrix play a role in development of metastatic cancer.

Using the technique of differential display polymerase chain reaction, it has been found that the cytokine, macrophage migration inhibitory factor (MIF), is one gene whose expression is altered in metastatic prostate cancer when compared to normal tissue (Meyer-Siegler, K. Hudon PB). Enhanced expression of macrophage migration inhibitory factor in prostatic adenocarcinoma metastases. *Urology* 48: 448–452, 1996.

MIF was first described thirty years ago and was designated as a cytokine, a chemical mediator which regulates cell growth by inducing the expression of specific target genes. The initial described function of MIF was as a regulator of inflammation and immunity. However, current research suggests an even greater role for MIF. It is expressed in the brain, and eye lens, is a delayed early response gene in fibroblasts, and it has been reported that this protein can be found in prostate tissues. MIF has been shown to be a pituitary, as well as macrophage cytokine and a critical mediator of septic shock. Recent studies also suggest that MIF may have an autocrine function for embryo development and is produced by the Leydig cells of the testes. Thus, it appears that this cytokine may play a fundamental role in cell growth regulation and possibly development.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a method for the diagnosis of adenocarcinoma and determining the potential metastatic of human cancer. The method is characterized by determining the relative levels of macrophage migration inhibitory factor within tumor cells. The method comprises the steps of obtaining tissue from an individual, extracting ribonucleic acid (RNA) and protein from the tissue and then determine the level of macrophage migration inhibitory factor (MIF). Alternatively, the MIF levels are determined directly in tissue samples.

The method of the invention includes the determination of a 166-bp DNA fragment that is not present in normal prostatic tissue.

In accordance with another embodiment of the invention, there is provided a kit for determining the relative levels of macrophage migration inhibitory factor within tumor cells. The kit comprises: a) a carrier compartmentalized to receive one or more container means therein, b) a first container means comprising oligonucleotide primer for binding cDNA, and c) a second container means comprising ingredients for differential display polymerase chain reaction for amplification of the cDNA.

It is therefore an object of the invention to provide a method for diagnosing cancer.

It is another object of the invention to provide a kit for detecting cancer.

It is a further object of the invention to provide a tumor marker for prostate cancer metastasis.

It is a still further object of the invention to distinguish histological tumors from clinical cancers.

These and other objects and advantages will be better understood from a reading of the description of the preferred embodiments and the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

According to the present invention, there is provided a method of determine the genetic changes associated with the development of metastic tumors, particularly, prostate cancer. The method includes the step of obtaining ribonucleic acid (RNA) from the tissue of an individual and then determine the relative levels of macrophage migration inhibitory factor. The method also provides for the determination of the level of migration inhibitory factor within the tissue sample.

The method further includes the step of determining the presence of a 166-bp DNA fragment.

The determination of the metastatic cancer according to one method is isolate RNA from tissue and using differential display polymerase chain reaction (DD-PCR) techniques with a primer amplifier to identify a 166-bp DNA fragment (D5k) that was not present in normal prostatic tissue. The D5k fragment displays 93% sequence similarity to nucleotides 662 to 845 of the human gene MIF. The sequence of D5k differs from that of human MIF at 11 nucleotides.

According to another embodiment of the invention, there is provided a kit for determining the levels of MIF within tumor cells. The kit comprises: a) a carrier compartmentalized to receive one or more container means therein, b) a first container means comprising oligonucleotide primer for binding cDNA, and c) a second container means comprising ingredients for differential display polymerase chain reaction for amplification of the cDNA.

Preferably, the primer is selected from the group consisting of T_{12} CT(5-dTTTTTTTTTTTTTCT-3') and 5'-TGTAGACCCT-3'.

It has now been found that MIF is expressed in the human prostate including pre-pubertal, pubertal, adult normal, benign hyperplastic, focal carcinoma, as well as metastatic prostate tissue samples. It has also been found to be expressed by both normal and cancerous glandular epithelial cells in the human breast, colon, and thyroid.